PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	Group Art Unit: 1648
Kotani *et al.*	)	
	)	Examiner: Chen
Serial No. 10/594,443	)	
	)	Confirmation No.: 5782
	)	
Filed: December 20, 2006	)	Atty. Dkt. No. 007123.00001

For: **COMPOSITION HAVING ANTITUMOR EFFECT**

DECLARATION OF TOSHIHIRO NAKAJIMA UNDER 37 C.F.R. § 1.132

U.S. Patent and Trademark Office
Randolph Building
401 Dulany Street
Alexandria, VA 22314

Sir:

I, **Toshihiro Nakajima**, declare as follows:

1. I am now **the CEO of GENOMIDEA Inc. which is one of the assignees of Serial No. 10/594,443" and a subsidiary of Anges MG, the other assignee,**. I was the CTO (Chief Technology Officer) from 1st June, 2002 until 6th March, 2008, and was a senior director of research, development & manufacturing department from 1st August, 2007 until 26th July, 2009. My *curriculum vitae* is attached as Exhibit 1.

2. This Declaration describes experiments which evaluated the efficacy of HVJ-E in tumor growth inhibition and survival prolongation in C57BL/6J mice bearing B16/BL6 murine melanoma cells.

3. A lyophilized formulation of HVJ-E prepared from human HEK293 host cells was used as a test substance in this study. The HVJ-E (40 or 400 HAU/mouse) was injected directly

into a tumor. The effect of injections 3 times a week for 1 week (Experiment A) and 3 times a week for 2 weeks (Experiment B) were examined.

4. The B16/BL6 murine melanoma cell line was purchased from Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University. The cells were grown in RPMI1640 (Invitrogen Inc.) supplemented with 10% fetal bovine serum, and cultured until the inoculation day.

5. On the inoculation day (Day 0), B16/BL6 cells in the log phase of growth were harvested by trypsinization and then resuspended in phosphate-buffered saline (PBS) to prepare the inoculatable cell suspension. Male 6-week-old C57BL/6J mice (The Charles River Laboratories Japan Inc) under general anesthesia by intra-peritoneal injection of Pentobarbital Na (Dainippon Sumitomo Pharma Co., Ltd.) were shaved dorsally using an electric animal shaver. The mice were inoculated intradermally with $5x10^5$ cells at the right dorsal region.

6. Four days after the inoculation (on day 4), the mice were divided into 6 groups (9 mice per group) which had almost uniform average size of tumors and allocated randomly to 3 groups for each of Experiment A and Experiment B (N=9).

7. The administration of HVJ-E in a 5% trehalose aqueous solution (40 or 400 HAU/mouse) or an equal volume of 5% trehalose aqueous solution (the vehicle) alone was performed on day 4, 6 and 8 in Experiment A, and on day 4, 6, 8, 11, 13 and 15 in Experiment B, respectively. On each injection day, the tumor-bearing mice were anesthetized with 2-3% inhaled isoflurane anesthesia (Forane: Abbott Japan Co., Ltd.) in an anesthesia box. The mice were taken out from the box and immediately given an intratumoral injection of 0.1 mL of HVJ-E solution or the vehicle alone using a 1 mL syringe (Terumo Corporation) and a 30G injection needle (Becton, Dickinson and Company).

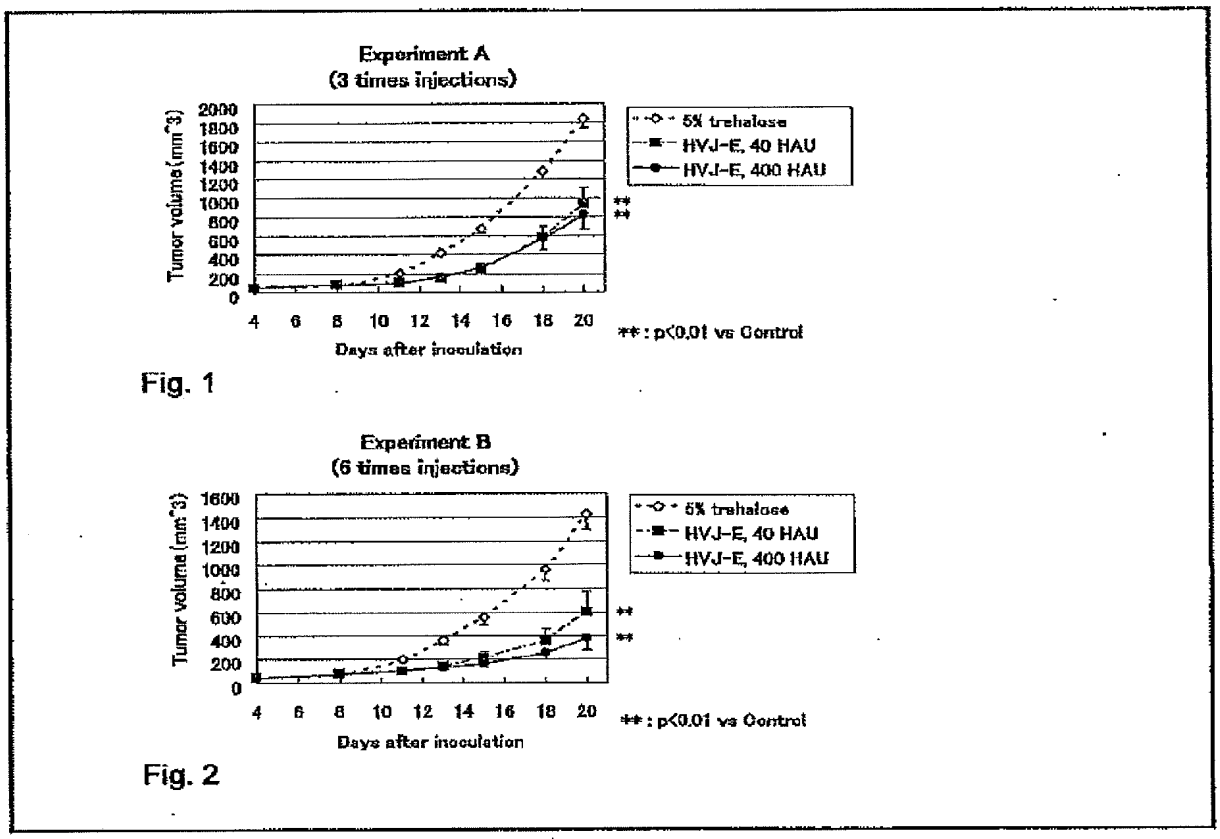
8. Tumor growth and change of body weight were monitored from the first day of administration (day 4) to day 36. The tumor volume in mm^3 was calculated by the formula: Volume = $(width)^2$ x length/2. The survival rate was monitored until the day when the death of the last survivor of the control group had been confirmed.

9. On day 11, a non-negligible difference in tumor volume was observed in the corresponding two groups in Experiment A and B which had already been treated three times with same dosage. Therefore, each of two groups were pooled before injection on day 11, then re-divided into the new two groups based on tumor volume, and randomly allocated to Experiments A and B.

10. Tumor volume on day 20 and survival until the final observation date were evaluated as the experimental endpoints. Day 20 was set as the limit of the period when the tumor size did not contribute to body weight under this experimental condition.

11. All statistical analyses were performed after excluding the mice that had died by the influence of intratumoral injection observed in the control group of Experiment B. Statistical significance was established at $P<0.05$. Tumor volume was analyzed by using Dunnett's multiple comparison test in each administration frequency (Experiment A or B). Mouse survival (vs. control) was analyzed by using Kaplan-Meier survival curve followed by Logrank test. P values were adjusted for multiple testing according to Holing method.

12. The tumor volume change (mean value and standard error) in each dosage group from the first injection day (day 4) to day 20 is shown in Fig. 1 for Experiment A (3 injections; N=9) and in Fig. 2 for Experiment B (6 injections; N=7-9). In both experiments, the remarkable tumor growth observed in the control group was significantly inhibited by both 40 and 400 HAU/mouse/injection of HVJ-E ($P<0.01$). During the same period, there was very little effect of HVJ-E dosing on weight growth.



13. The survival curves on cancer-related death is shown in Fig. 3 for Experiment A (3 injections; N=9) and in Fig. 4 for Experiment B (6 injections; N=7~9). In Experiment A, survival was significantly prolonged by 40 HAU/mouse/injection ($P<0.05$), but not by 400 HAU/mouse/injection, of HVJ-E. In Experiment B, survival was significantly prolonged by both dosages of HVJ-E ($P<0.05$ in 40 HAU/mouse/injection; $P<0.01$ in 400 HAU/mouse/injection).

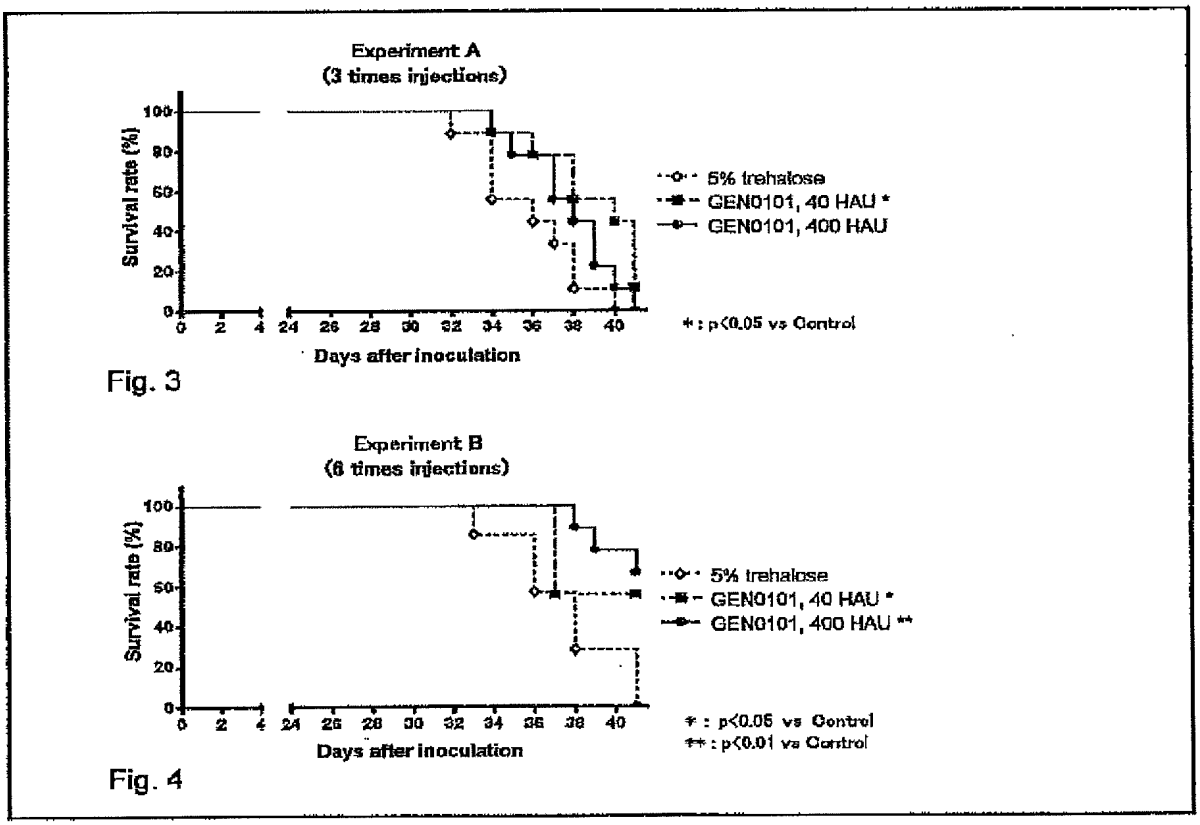


Fig. 3

Fig. 4

14. As described above, intratumoral injections of HVJ-E resulted in a significant level of tumor growth inhibition and prolongation of survival in a mouse melanoma model, indicating the potential of HVJ-E as a new therapeutic agent for melanoma.

15. All statements I made in this declaration of my own knowledge are true. I believe all statements made on information and belief to be true. I made these statements with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the patent.

Dated: 5th January, 2010

Toshihiro NAKAJIMA

EXHIBIT 1

Curriculum Vitae

Toshihiro Nakajima, Ph.D.
Chief Executive Officer (CEO)
GenomIdea Inc.

Toshihiro Nakajima, Ph.D., a chief executive officer (CEO) of GenomIdea Inc. is developing the non-virus vector (HVJ-envelope vector) for cancer immune therapy and DNA vaccine for infectious diseases. GenomIdea Inc (subsidiary of AnGes MG) is an Osaka University-derived campus bioventure company and focuses on the development of the HVJ-envelope vector for gene therapy.

Dr. Nakajima studied under Professor Tadamitsu Kishimoto's supervision and received his Ph.D. in immunology from Osaka University. He completed his post-doctoral training under Dr. Shizuo Akira's supervision at Institute for Molecular and Cellular Biology in Osaka University.

Prior to joining GenomIdea, Dr. Nakajima joined Medgene bioscience (AnGes MG later on) in 2000, and was a general manager of the R&D division of AnGes MG.

Preceding AnGes MG, Dr Nakajima was a researcher of Shionogi Institute of Medical Science. He was also served on the research staff of the DNAVEC Research Institute (Tsukuba, Ibaraki) and developed a nonpathogenic lentivirus-based vector (pseudotype SIV-based vector with VSV or HVJ envelope proteins).

EXHIBIT 1

Toshihiro Nakajima, Ph.D.
Chief Executive Officer (CEO)
GenomIdea Inc.
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Fax : +81-72-751-1176
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Education

1993 Ph. D., Institute for Molecular and Cellular Biology in Osaka University
"Phosphorylation at threonine-235 by a ras-dependent mitogen-activated protein kinase cascade is essential for transcription factor NF-IL6."

1993 post-doctoral training under Dr. Shizuo Akira's supervision at Institute for Molecular and Cellular Biology in Osaka University

Employment

1993-1997 Shionogi Institute for Medical Science
- Identification of the novel chemokine (eotaxin) and it's receptor (CCR3)
- Characterization of the T-cell tropic HIV receptor (fusin/CXCR4)

1997-1999 DNAVEC Research Inc. (temporary transferred from Shionogi)
- Development of the SIVagm-besed vectors for gene therapy (pseudotyped with VSV or HVJ envelope proteins)

1999-2000 Shionogi Research Laboratories

2000-present MedGene Bioscience Co., Ltd. (AnGes MG Inc. later on)

2002.7-2007.7 GenomIdea Inc. (Chief Technology Officer: CTO)

2007.8-2007.10 GenomIdea Inc. (CTO and senior director of Research, Development & Manufacturing Department)

2007.10-2008.3 GenomIdea Inc. (Chief Executive Officer: CEO, CTO and senior director of Research, Development & Manufacturing Department)

2008.3-2009.7 GenomIdea Inc. (CEO and senior director of Research, Development & Manufacturing Department)

2009.7-present GenomIdea Inc. (CEO)
- Development of the HVJ-envelope (HVJ-E) vector for the cancer immune therapy
- Development of the HVJ-envelope vector-based DNA vaccine for infectious diseases